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NOTICE of CHANGE dated 28/11/2023

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«BCR-ABL P210 ELITe MGB® Kit»
Ref. RTSG07PLD210

This new revision of the Instruction for Use (IFU) contains the following changes:

- *Updated transport and storage conditions for primary sample*

Composition, use and performance of the product remain unchanged.

PLEASE NOTE

	LA REVISIONE DI QUESTO IFU E' COMPATIBILE ANCHE CON LA VERSIONE PRECEDENTE DEL KIT
	THE REVIEW OF THIS IFU IS ALSO COMPATIBLE WITH THE PREVIOUS VERSION OF THE KIT
	CET IFU MIS A JOUR ANNULE ET REMPLACE ET EST PARFAITEMENT COMPATIBLE AVEC LA VERSION PRECEDENTE DU KIT
	LA REVISIÓN DE ESTE IFU ES COMPATIBLE TAMBIÉN CON LA VERSIÓN ANTERIOR DEL KIT
	A REVISÃO DO ESTE IFU ÉTAMBÉM COMPATÍVEL COM A VERSÃO ANTERIOR DO KIT
	DIE REVIEW VON DIESER IFU IST KOMPATIBLE MIT DER VORIGE VERSION VON DEM TEST-KIT

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BCR-ABL P210 ELITe MGB® Kit

reagents for RNA reverse transcription and cDNA Real Time amplification

REF RTSG07PLD210 IVD -20°C

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INTENDED USE

The **«BCR-ABL P210 ELITe MGB® Kit»** product is a qualitative and quantitative, reverse transcription and amplification of nucleic acids assay for the **detection of the mRNA of the BCR-ABL rearrangement, t(9;22) translocation, *Philadelphia* chromosome, variant P210 (P210)** and for the **quantification of the mRNA of P210 compared with the mRNA of the gene codifying the kinase protein Abelson (ABL)** in total RNA samples extracted from lympho-monocyte suspensions and leukocyte suspensions from clinical samples of peripheral blood or bone marrow.

The product is intended for use, alongside clinical data and other laboratory tests, as an aid in the diagnosis and in the monitoring of cases of chronic myeloid leukaemia (CML), acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL) positive for the P210 marker.

The results obtained with this product can be aligned to the International Scale (IS) by a conversion factor that can be calculated using the product **«PHILADELPHIA P210 RNA Reference»**, manufactured by ELITechGroup S.p.A. and calibrated against the "1st World Health Organization (WHO) International Genetic Reference Panel for quantitation of BCR-ABL translocation by RQ-PCR".

ASSAY PRINCIPLE

The assay consists of a reverse transcription and a real time amplification reaction (one-step method) with a programmable thermostat provided with a fluorescence detection optical system (real time amplification thermal cycler).

For each extracted RNA sample, the assay involves **a duplicate specific reaction for a P210 mRNA region** (target) and **a duplicate specific reaction for an ABL mRNA region** (control).

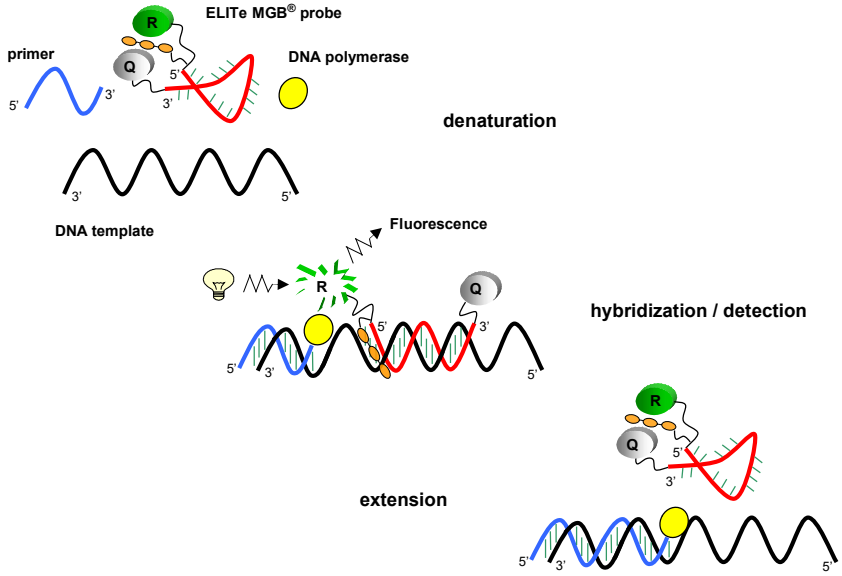
The P210 cDNA specific probe with ELITe MGB® technology, labelled with FAM fluorophore, is activated when hybridizes with the specific product of the P210 cDNA amplification reaction.

The ABL cDNA specific probe with ELITe MGB® technology, labelled with FAM fluorophore, is activated when hybridizes with the specific product of ABL cDNA amplification reaction.

As the specific product of the amplification reaction increases, the fluorescence emission increases and is measured and recorded by the instrument. The processing of the data determines the presence and the titre of P210 and ABL mRNA in the starting sample.

The assay is validated with the systems described in this user manual.

The following picture shows the mechanism of activation and fluorescence emission of the ELITe MGB® technology probe. Note that the probe is not hydrolyzed during the amplification cycles.



PRODUCT DESCRIPTION

The **«BCR-ABL P210 ELITe MGB® Kit»** product provides the following components:

- **P210 PreMix**

A mixture of oligonucleotides, specific for P210 reverse transcription and real time amplification, in a stabilized solution, **aliquoted into a test tube** (WHITE cap), containing **270 µL** of solution, sufficient for at least **36 tests** in association with **«ELITe InGenius®»** and **50 tests** in association with other systems.

Primer oligonucleotides and the specific probe for P210 (stabilized by MGB® group, labelled by FAM fluorophore and quenched by a non-fluorescent molecule), which are specific for a region of the mRNA generated by **BCR-ABL rearrangement (variant P210 b3a2 and variant P210 b2a2)**.

The reaction mixture provides AP593 fluorophore, used instead of ROX or CY5, as passive reference for fluorescence normalisation.

- **ABL PreMix**

A mixture of oligonucleotides, specific for ABL reverse transcription and real time amplification, in a stabilized solution, **aliquoted into a test tube** (NEUTRAL cap), containing **270 µL** of solution, sufficient for at least **36 tests** in association with **«ELITe InGenius®»** and **50 tests** in association with other systems.

Primer oligonucleotides and the specific probe for ABL (stabilized by MGB® group, labelled by FAM fluorophore and quenched by a non fluorescent molecule) which are specific for a region of the mRNA of the human gene encoding **ABL (exons a2a3)**.

The mixture provides AP593 fluorophore, used instead of ROX or CY5, as passive reference for fluorescence normalisation.

- **PCR MasterMix**

A mixture of optimized and stabilized reagents for reverse transcription and real time amplification **aliquoted into 2 test tubes** (NEUTRAL cap). Each tube contains **820 µL** of solution, which is sufficient for at least **36 tests** in association with **«ELITe InGenius®»** and **50 tests** in association with other systems.

The mixture provides the buffer, magnesium chloride, the nucleotide triphosphates and the hot start Taq DNA polymerase enzyme.

- **RT EnzymeMix**

A mixture of optimized and stabilized reagents for reverse transcription, **aliquoted into 2 test tubes** (cap with BLACK insert). Each tube contains **20 µL** of solution, sufficient for at least **36 tests** in association with **«ELITe InGenius®»** and **50 tests** in association with other systems.

The mixture provides the reverse transcriptase enzyme.

The product enables **18 duplicate determinations for the mRNA of P210** and **18 duplicate determinations for the mRNA of ABL in association with ELITe InGenius**, including standards and controls.

The product enables **25 duplicate determinations for the mRNA of P210** and **25 duplicate determinations for the mRNA of ABL in association with** 7300 Real Time PCR System, 7500 Fast Dx Real-Time PCR Instrument and 7900 Real-Time PCR System, including standards and controls, i.e. maximum number of **19 clinical samples** in one session (under optimal conditions of use).

BCR-ABL P210 ELITe MGB® Kit
reagents for RNA reverse transcription and cDNA Real Time amplification

REF **RTSG07PLD210**

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Hazard classification
P210 PreMix	Primer/probe oligonucleotides mixture WHITE cap	**1 x 270 µL**	-
ABL PreMix	Primer/probe oligonucleotides mixture NEUTRAL cap	**1 x 270 µL**	-
PCR MasterMix	mixture of reagents for reverse transcription and real time amplification NEUTRAL cap	**2 x 820 µL**	-
RT EnzymeMix	Reverse transcriptase cap with BLACK insert	**2 x 20 µL**	-

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench microcentrifuge (12,000 - 14,000 RPM).
- Sterile micropipettes and tips with aerosol filter or positive displacement (2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- Molecular biology grade water.
- Sarstedt 2.0 mL skirted tube with screw-cap (Sarstedt Ref. 72.694.005).
- Polypropylene 1.5 mL microtubes for molecular biology.
- Programmable thermostat with optical fluorescence detection system 7300 Real Time PCR System, 7500 Fast Dx Real-Time PCR Instrument or 7900 Real-Time PCR System calibrated following manufacturer's instructions.

OTHER PRODUCTS REQUIRED

The reagents for the extraction of RNA from samples, amplification microplates and known-quantity DNA standards **are not** included in this product.

For automatic sample analysis with the instrument **«ELITe InGenius»** (ELITechGroup S.p.A., ref. INT030) the following generic products are required: the extraction cartridges **«ELITe InGenius® SP RNA»** (ELITechGroup S.p.A., ref. INT034SPRNA), the **«ELITe InGenius DNase I»** (ELITechGroup S.p.A. INT034DNASE), the **«Dnase Tube Adapter Kit»** (ref. G6431-000), the consumables for extraction and amplification of nucleic acids from biological samples **«ELITe InGenius® SP 200 Consumable Set»** (ELITechGroup S.p.A, ref. INT032CS), **«ELITe InGenius® Waste Box»** (ELITechGroup S.p.A, ref. F2102-000), **«ELITe InGenius® PCR Cassette»** (ELITechGroup S.p.A, ref. INT035PCR) and **«300 µL Filter Tips Axygen»** (Axygen BioScience Inc., CA, USA, ref. TF-350-L-R-S).

For automatic RNA extraction, amplification and interpretation of sample analysis, the instrument **«ELITe InGenius»** (ELITechGroup S.p.A., ref. INT030) and the following specific Assay protocols (ELITechGroup S.p.A.), are required:

for the calibrators **«BCR-ABL P210 ELITe_STD_P210»** and **«BCR-ABL P210 ELITe_STD_ABL»**,
for the positive control of amplification **«BCR-ABL P210 ELITe_PC»**,
for negative control of amplification **«BCR-ABL P210 ELITe_NC»**,
for samples analysis **«BCR-ABL P210 ELITe_PBL_200_100».**

For RNA extraction from samples to be analyzed, use a laboratory validated generic product, such as the **«Maxwell® CSC»** (Promega, code AS6000) automatic extraction system with **Maxwell® CSC RNA Blood Kit** reagents (Promega, code AS1410) or other equivalent products.

When 7300 Real-Time PCR System is used, it is required the use of generic product: **«MicroAmp™ Optical 96-Well Reaction Plate»** (Life Technologies, ref. N8010560), microplates with 0.2 mL wells and adhesive sealing sheets for real time amplification.

When 7500 Fast Dx Real-Time PCR Instrument is used, it is required the use of generic product: **«MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL»** (Life Technologies, ref. 4346906), microplates with 0.1 mL wells and adhesive sealing sheets for real time amplification.

For detection and quantification of P210 mRNA and ABL mRNA, the product **«BCR-ABL P210 - ELITe Positive Control»** (ELITechGroup S.p.A., ref. CTRG07PLD210), positive control of plasmid DNA, is required.

For detection and quantification of P210 mRNA and ABL mRNA, the product **«BCR-ABL P210 ELITe Standard»** (ELITechGroup S.p.A., code STDG07PLD210), five dilutions of known-quantity plasmid DNA to obtain P210 and ABL standard curves, is required.

The product **«PHILADELPHIA P210 RNA Reference»** (ELITechGroup S.p.A., code SPG07-210), four mixtures of total RNA at known-quantity for conversion factor calculation, is recommended in order to express the results in International Scale (IS) as per "1st World Health Organization (WHO) International Genetic Reference Panel for quantitation of BCR-ABL translocation by RQ-PCR".

For blood pre-treatment, use a laboratory validated generic product, such as the Cell Lysis Solution (Promega, Ref. A7933), RNA Lysis Buffer (Promega, Ref. Z3051) and Thioglycerol (Promega, Ref. A208B-C) or equivalent reagents (such as Solution A (Promega, Ref. MC130A), Solution B (Promega, Ref. MC131A) and Thioglycerol (Promega, Ref. MC132A).

WARNINGS AND PRECAUTIONS

This product is exclusively designed for *in-vitro* use.

General warnings and precautions

Handle and dispose of all biological samples as if they were able to transmit infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite or autoclaved for one hour at 121°C before disposal.

Handle and dispose of all reagents and all materials used to carry out the assay as if they were able to transmit infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be handled and disposed of in compliance with adequate safety standards. Disposable combustible material must be incinerated. Liquid waste containing acids or bases must be neutralised before disposal.

Wear suitable protective clothes and gloves and protect eyes and face.
Never pipette solutions by mouth.
Do not eat, drink, smoke or apply cosmetic products in work areas.
Carefully wash hands after handling samples and reagents.
Dispose of leftover reagents and waste in compliance with the regulations in force.
Carefully read all instructions provided in the product before running the assay.
While running the assay, follow the instructions provided in the product.
Do not use the product after the indicated expiry date.
Only use the reagents provided in the product and those recommended by the manufacturer.
Do not mix reagents from different batches.
Do not use reagents from other manufacturers.

Warnings and precautions for molecular biology

Molecular biology procedures, such as nucleic acid extraction, reverse transcription, amplification and detection, require qualified and trained staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained within the samples or sample contamination by amplification products.

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When amplification session is manually setup, it is necessary to have available separate areas for the extraction / preparation of amplification reactions and for the amplification / detection of amplification products. Never introduce an amplification product in the area designated for extraction / preparation of amplification reactions.

When amplification session is manually setup, it is necessary to have available lab coats, gloves and tools which are exclusively used for the extraction / preparation of the amplification reactions and for the amplification / detection of amplification products. Never transfer lab coats, gloves or tools from the area designated for the amplification / detection of amplification products to the area designated for the extraction / preparation of the amplification reactions.

The samples must be exclusively used for this type of analysis. Samples must be handled under a laminar airflow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively used for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases, and free from DNA and RNA.

The reagents must be handled under a laminar airflow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. The pipettes used to handle the reagents must be exclusively used for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNAses and RNAses, and free from DNA and RNA.

Amplification products must be handled in such a way as to reduce dispersion into the environment in order to avoid the possibility of contamination. The pipettes used to handle amplification products must be exclusively used for this purpose.

Warnings and precautions specific for the components

- **P210 PreMix**
  The **P210 PreMix** must be stored at -20°C in the dark.
  The **P210 PreMix** can be frozen and thawed for no more than **six times**: further freezing / thawing cycles may reduce product performance.
- **ABL PreMix**
  The **ABL PreMix** must be stored at -20°C in the dark.
  The **ABL PreMix** can be frozen and thawed for no more than **six times**: further freezing / thawing cycles may reduce product performance.
- **PCR MasterMix**
  The **PCR MasterMix** must be stored at -20°C.
  The **PCR MasterMix** can be frozen and thawed for no more than **six times**: further freezing / thawing cycles may reduce product performance.
- **RT EnzymeMix**
  The **RT EnzymeMix** must be stored at -20°C.
  The **RT EnzymeMix** must not be exposed to temperatures higher than -20 °C for more than 10 minutes for no more than **six times**.

ELITe InGenius®

SAMPLES AND CONTROLS

Samples

This product must be used with the following clinical samples:

Peripheral blood collected in EDTA or sodium citrate

The peripheral blood collected in EDTA or sodium citrate, used for lymphomonocyte and leukocyte suspensions preparation for RNA extraction, must be collected according to laboratory guidelines, transported and stored at room temperature (+21 ±5 °C) for a maximum of 24 hours.

Do not freeze peripheral blood in order to prevent degradation of RNA.

When starting with peripheral blood it is advisable to separate leukocyte according to laboratory guidelines or following indications.

Transfer 10 – 14 mL of fresh peripheral blood collected in EDTA or sodium citrate into a 15 mL tube after mixing it thoroughly by inversion. Centrifuge for 10 minutes at 3000 RCF; add 5 mL of Cell Lysis Solution (Promega, Ref. A7933) into a new 15 mL tube; with a 1 mL pipette, remove the buffy-coat obtained after centrifugation and transfer it to the 15 mL tube containing the lysis solution; aspirate and release until the cells are inside the tube and the pipette is free of material; incubate at room temperature for 10 minutes and mix by inversion (NO VORTEX) at least 3-4 times; centrifuge at 3000 RCF for 10 minutes.

Note: the ideal amount of white cells, is represented, in 1:1 scale, in the following picture

Remove the supernatant and resuspend in 2 mL of Cell Lysis Solution by transferring it into a 2 mL tube; centrifuge again for about 2 minutes at 3000 RCF; carefully remove the supernatant (attention to remove traces of red cells above the with cells pellet) and resuspend the pellet in 200 µL of Lysis Solution (1 mL of RNA Lysis Buffer, Promega, Ref. Z3051 + 20 µL of 1-Thioglycerol, Promega, Ref. A208B-C).

Note: when nucleic acid extraction is carried out with the **ELITe InGenius** and with **ELITe InGenius® Software** version 1.3 (or later equivalent versions), use the extraction protocols **BCR-ABL P210 ELITe_PBL_200_100**. This protocol processes 200 µL of sample and elutes the nucleic acids in 100 µL.

Interfering substances

The extracted RNA must not contain heparin, haemoglobin, Ficoll®, ethanol or 2-propanol in order to prevent inhibition and the possibility of frequent invalid results.

Quantities of RNA more than 2.0 µg per reaction could inhibit the reverse transcription reaction and the real time amplification.

Quantities of human genomic DNA higher than 100 ng per reaction in the RNA extracted from the sample could inhibit the reverse transcription reaction and the real time amplification.

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PROCEDURE

The procedure to use the **BCR-ABL P210 ELITe MGB® Kit** with the system **ELITe InGenius** consists of three steps:

- Verification of the system readiness,
- Set up of the session,
- Review and export of results.

System readiness verification

Before starting the session, referring to the instrument documentation, it is necessary to:
- switch on the **ELITe InGenius** and select the mode "**CLOSED**",
- verify that the Calibrators (**BCR-ABL P210 Q-PCR Standard**) were run, approved and are not expired (Status) in association with the amplification reagent lot to be used. If there are not amplification Calibrators approved or valid, run them as described in the following paragraphs,
- verify that the amplification Controls (Controls, **BCR-ABL P210 Positive Control, BCR-ABL P210 Negative Control**) were run, approved and are not expired (Status) in association with the amplification reagent lot to be used. If there are not amplification Controls approved or valid, run them as described in the following paragraphs,
- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by ELITechGroup S.p.A. These IVD protocols were specifically validated with ELITe MGB® kits, **ELITe InGenius** instrument and the cited matrix.

The Assay Protocol available for sample testing with the product **BCR-ABL P210 ELITe MGB® Kit** is described in the table below.

Assay protocol for BCR-ABL P210 ELITe MGB® kit			
Name	**Matrix**	**Report unitage**	**Characteristics**
BCR-ABL P210 ELITe_PBL_200_100	Peripheral Blood Leukocyte	%P210	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Sonication: NO Internal Control: NO PCR Mix volume: 20 µL Sample PCR input volume: 10 µL

If the assay protocol of interest is not in the system, contact your local ELITechGroup Customer Service.

Setup of the session

The product **BCR-ABL P210 ELITe MGB® Kit** in association to the **ELITe InGenius** can be used in in order to perform:

A. Integrated run (Extract + PCR),
B. Amplification run, (PCR only),
C. Calibration run (PCR only),
D. Amplification run for Positive and Negative Control (PCR only).

All the parameters needed for the session are included in the Assay protocol available on the instrument and are automatically recalled when the Assay protocol is selected.

Note: The **ELITe InGenius** system can be linked to the "Location Information Server" (LIS) through which it is possible to send the work session information. Refer to the instrument user's manual for more details.

Before starting the session, it is mandatory to do the following:

1. Thaw for 30 minutes at room temperature (+18 / 25 °C) the **P210 PreMix** (WHITE cap) and the **ABL PreMix** (NEUTRAL cap) test tubes needed for the session, remembering that the content of each test tube is enough for **36 reactions**. Mix by vortexing for 10 seconds three times and centrifuge the tubes for 5 seconds to bring the content to the bottom and keep in ice.

2. Thaw for 30 minutes at room temperature (+18 / 25 °C) the **PCR MasterMix** (NEUTRAL cap) test tubes needed for the session, remembering that the content of each test tube is enough for **36 reactions**. Mix by vortexing for 10 seconds three times and centrifuge the tubes for 5 seconds to bring the content to the bottom and keep in ice.

3. Take the **RT EnzymeMix** (cap with BLACK insert) tubes necessary for the session remembering that the content of each tube is sufficient to set up **36 reactions**. Gently shake the tubes, centrifuge for 5 seconds to bring the contents to the bottom and keep in ice.

Note: The **RT EnzymeMix** should not be exposed to temperatures above -20 °C for more than 10 minutes.

4. Prepare one 2 mL tube with screwed cap (Sarstedt Ref. 72.694.005, not included in the kit) for the **complete reaction mixture** and mark it in a recognizable manner with a permanent marker.

5. Calculate the volumes of the three components provided by kit that are needed for preparing the **complete reaction mixture:**

a. For the Calibration follow the table below:

Target	Number of samples	PreMix	PCR MasterMix	RT EnzymeMix
P210	5	30 µL	90 µL	0.9 µL
ABL	3	20 µL	60 µL	0.6 µL

b. for Controls and samples follow the table below:

Number of samples	P210 or ABL PreMix	PCR MasterMix	RT EnzymeMix
1	15 µL	45 µL	0.5 µL
2	25 µL	75 µL	0.8 µL
3	40 µL	120 µL	1.2 µL

6. Prepare the **complete reaction mixture** by adding into the dedicated 2 mL tube the calculated volumes of the three components.

7. Mix by **vortexing at low speed** for 10 seconds three times, centrifuge the tube for 5 seconds to bring the content to the bottom and keep in ice.

Note: The **complete reaction mixture** should be used within **5** hours when kept on board in the refrigerated block. The complete reaction mixture **cannot** be stored. This time allows to carry out 1 work session of 3.5 hours and to start a second work session.

The main operations for setting the four types of travel are described below.

A. Integrated run

To set up the integrated run starting from pre-treated samples, carry out the steps below following the GUI:

1. Select “Perform Run” from the “Home” screen.
2. Ensure that the “Extraction Input Volume” is 200 µL and that the “Extracted Elute Volume” is 100 µL.
3. For each Track of interest fill in the “SampleID” (SID) by typing or by scanning the sample barcode.
4. Select the assay protocol to be used in the “Assay” column (i.e. BCR-ABL P210 ELITe_PBL_200_100).
5. Ensure that the “Protocol” displayed is: “Extract + PCR”.
6. Ensure the sample loading position in the “Sample Position” column is “Extraction Tube (bottom row)”. Click “Next” to continue the setup.
7. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
8. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.

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9. Load the “PCR Cassette”, the “ELITe InGenius SP RNA” extraction cartridges and the ”ELITe InGenius DNase I“, all the required consumables and the samples to be extracted in the positions specified in step 8, following the GUI instruction. Click “Next” to continue the setup.
10. Close the instrument door.
11. Press “Start” to start the run.

After process completion, the **ELITe InGenius** allows the user to view, approve, store the results and to print and save the report.

Note: At the end of the run the remaining Extracted Sample in the “Elution tube” must be removed from the instrument, capped, identified and stored at -20 °C for one month. Avoid spilling Extracted Sample.

Note: At the end of the run the PCR Cassettes with the reaction products and the consumables must be removed from the instrument and disposed of without producing environmental contaminations. Avoid spilling the reaction products.

Note: At the end of the run, the **complete reaction mixture** can be kept on board in the refrigerated block taking into account the maximum time of 5 hours.

B. Amplification run

To set up the amplification run starting from extracted RNA, carry out the following steps as per GUI:

1. Select “Perform Run” from the “Home” screen.
2. Even if no extraction will be carried out, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 100 µL.
3. For each Track of interest fill in the SID by typing or by scanning the sample barcode.
4. Select the assay protocol to be used in the “Assay” column (i.e. BCR-ABL P210 ELITe_PBL_200_100).
5. Select “PCR Only” in the “Protocol” column.
6. Ensure the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”. Click “Next” to continue the setup.
7. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
8. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
9. Load the “PCR Cassettes” and the extracted Nucleic Acid samples following the GUI instruction. Click “Next” to continue the setup
10. Close the instrument door.
11. Press “Start” to start the run.

After process completion, the **ELITe InGenius** allows the user to view, approve, store the results and to print and save the report.

Note: At the end of the run the remaining Extracted Sample in the “Elution tube” must be removed from the instrument, capped, identified and stored at -20 °C for one month. Avoid spilling Extracted Sample.

Note: At the end of the run the PCR Cassettes with the reaction products and the consumables must be removed from the instrument and disposed of without producing environmental contaminations. Avoid spilling the reaction products.

Note: At the end of the run, the **complete reaction mixture** can be kept on board in the refrigerated block taking into account the maximum time of 5 hours.

C. Calibration run

To set up the Calibration run for Q-PCR Standards, carry on the steps below following the GUI:

1. Thaw a tube of each BCR-ABL P210 Q - PCR Standard levels for P210 calibration (Cal1: BCR-ABL Q-PCR Standards 10^1, Cal2: BCR-ABL Q-PCR Standards 10^2, Cal3: BCR-ABL Q-PCR Standards 10^3, Cal4: BCR-ABL Q-PCR Standards 10^4, Cal5: BCR-ABL Q-PCR Standards 10^5). Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
2. Thaw another tube of BCR-ABL P210 Q - PCR Standard 10^5, 10^4 and 10^3 for ABL calibration (Cal3: BCR-ABL Q-PCR Standards 10^3, Cal4: BCR-ABL Q-PCR Standards 10^4, Cal5: BCR-ABL Q-PCR Standards 10^5). Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Select “Perform Run” from the “Home” screen.
4. Even if no extraction will be carried out, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 100 µL.
5. For P210 calibration, select the Assay Protocol “BCR-ABL P210 ELITe_STD_P210” in the “Assay” column and fill in the lot number and expiry date of **BCR-ABL P210 Q-PCR Standard**.
6. For ABL calibration, select the Assay Protocol “BCR-ABL P210 ELITe_STD_ABL” in the “Assay” column and fill in the lot number and expiry date of **BCR-ABL P210 Q-PCR Standard**.
7. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
8. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
9. Load the “PCR Cassettes” and the **BCR-ABL P210 Q-PCR Standard** tubes following the GUI instruction. Click “Next” to continue the setup.
10. Close the instrument door.
11. Press “Start” to start the run.

After process completion, the **ELITe InGenius** allows the user to view, approve, store the results and to print and save the report.

Note: At the end of the run the remaining **BCR-ABL P210 Q-PCR Standard** must be removed from the instrument, capped and stored at -20 °C.

Note: At the end of the run the PCR Cassettes with the reaction products and the consumables must be removed from the instrument and disposed of without producing environmental contaminations. Avoid spilling the reaction products.

Note: At the end of the run, the **complete reaction mixture** can be kept on board in the refrigerated block taking into account the maximum time of 5 hours.

D. Amplification run for Positive Control and Negative Control

To setup the amplification run for Positive Control and Negative Control, carry out the following steps as per GUI:

1. Thaw BCR-ABL P210 - ELITe Positive Control tube for the session. Each tube is sufficient for 2 sessions. Mix gently, spin down the content for 5 seconds.
2. Transfer at least 80 µL of molecular biology grade water to an “Elution tube”, provided with the ELITe InGenius SP 200 Consumable Set.
3. Select “Perform Run” from the “Home” screen.
4. Even if no extraction will be carried out, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 100 µL.

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5. In the Track of interest, select the Assay protocol to be used in the “Assay” column.
6. For the positive control, select the Assay Protocol “BCR-ABL P210 ELITe_PC” in the “Assay” column and fill in the lot number and expiry date of BCR-ABL P210 Positive Control.
7. For the negative control, select the Assay Protocol “BCR-ABL P210 ELITe_NC” in the “Assay” column and fill in the lot number and expiry date of the molecular biology grade water.
8. Click “Next” to continue the setup.
9. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
11. Load the “PCR cassettes”, the BCR-ABL P210 Positive Control tube and the Negative Control tube following the GUI instruction. Click “Next” to continue the setup.
12. Close the instrument door.
13. Press “Start” to start the run.

After process completion, the **ELITe InGenius** allows the user to view, approve, store the results and to print and save the report.

Note: At the end of the run the remaining **BCR-ABL P210 Positive Control** must be removed from the instrument, capped and stored at -20 °C. The remaining Negative Control must be disposed.

Note: At the end of the run the PCR Cassettes with the reaction products and consumables must be removed from the instrument and disposed of without environmental contaminations. Avoid spilling the reaction products.

Note: At the end of the run, the **complete reaction mixture** can be kept on board in the refrigerated block taking into account the maximum time of 5 hours.

Review and approval of results

At the end of the run, the “Results Display” screen is automatically shown. In this screen the sample / Calibrator / Control results and the information regarding the run are shown. From this screen is possible to approve the result, print or save the reports (“Sample Report” or “Track Report”). Refer to the instrument user’s manual for more details.

Note: The **ELITe InGenius** can be linked to the “Laboratory Information Server” (LIS) through which it is possible to send the work session results to the laboratory data centre. Refer to the instrument user’s manual for more details.

The **ELITe InGenius** generates results using the **BCR-ABL P210 ELITe MGB® Kit** through the following procedure:

A. Validation of Calibration curve,
B. Validation of amplification Positive Control and Negative Control results,
C. Validation of sample results,
D. Sample result reporting.

A. Validation of Calibration curve

The fluorescence signals emitted by the probe for P210 (Channel 1 "P210") in the Calibrator amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocol “BCR-ABL ELITe_STD_P210”.

The fluorescence signals emitted by the probe for ABL (Channel 1 "ABL") in the Calibrator amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocol “BCR-ABL ELITe_STD_ABL”.

The P210 and ABL Calibration curves, specific for the amplification reagent lot, is stored in the database (Calibration). It can be viewed and approved by personnel qualified as “Administrator” or “Analyst” following the GUI instructions.

The Calibration curves, specific for the amplification reagent lot, will expire **after 60 days**.

Note: If the Calibration curve does not meet the acceptance criteria, the “Failed” message is shown on the “Calibration” screen and it is not possible to approve the curve. The Calibrator amplification reactions must be repeated.

Note: If the Calibration Curve is run together with samples and its result is invalid, the entire session is invalid. In this case, the amplification of all samples must be repeated too.

B. Validation of amplification Positive Control and Negative Control results

The fluorescence signals emitted by the probe for P210 (Channel 1 "P210") in the Positive Control and Negative Control amplification reaction are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocol “BCR-ABL P210 ELITe_PC” and “BCR-ABL P210 ELITe_NC”.

The amplification Positive Control and Negative Control results, specific for the lot of amplification reagent used, are recorded in the database (Controls). They can be viewed and approved by personnel qualified as “Administrator” or “Analyst”, following the GUI instructions.

The amplification Positive Control and Negative Control results, specific for the amplification reagent lot, will expire **after 15 days**.

The results of Positive Control and Negative Control amplification are used by the instrument software to calculate and setup the “Control Charts”. Four Positive Control and Negative Control results, from 4 different runs are requested to set up the “Control Chart”. After that, the results of Positive control and Negative Control are used for monitoring the amplification step performances. Refer to the user’s manual of the instrument for more details.

Note: If the amplification Positive Control or Negative Control result does not meet the acceptance criteria, the “Failed” message is shown on the “Controls” screen and it is not possible to approve it. In this case, the amplification Positive Control or Negative Control reaction must be repeated.

Note: If the Positive Control or Negative Control is run together with samples to be tested and its result is invalid, the entire session is invalid. In this case, the amplification of all samples must be repeated too.

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C. Validation of Samples results

The fluorescence signals emitted by the probe for P210 (Channel 1 "P210") and by the probe for ABL (Channel 1 "ABL") in the sample amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the Assay Protocol BCR-ABL P210_PBL_200_100.

Results are shown in the reports generated by the instrument ("Result Display").

The sample run can be approved when the three conditions reported in the table below are met.

1) Calibration curve	**Status**
BCR-ABL P210 Q-PCR Standard	APPROVED
2) Positive Control	**Status**
BCR-ABL P210 Positive Control	APPROVED
3) Negative Control	**Status**
BCR-ABL P210 Negative Control	APPROVED

For each sample, the assay result is automatically interpreted by the system as established by the **ELITe InGenius software** algorithm and the Assay protocol parameters and explains in the following paragraph.

In case of the amplification reactions of each **sample**, **P210 Ct** values are used to detect and quantify the presence of target mRNA, while **ABL Ct** values are used to detect and quantify the presence of control mRNA (extraction validation and target normalization).

The **P210 Ct** and **ABL Ct** values in the amplification reactions of each **sample** and the **Standard Curves** are used to calculate the **Quantity of mRNA** of P210 and ABL present in the amplification reactions of the samples. Then the **Quantities of mRNA** of P210 and ABL are used to calculate the **percentage of P210** mRNA copies normalized to ABL mRNA copies (**%P210**).

The possible result messages of a Sample are listed the table below.

Result of Sample run	**Interpretation**
P210:percentage is x.xxxx%	**P210 RNA was detected.** Calculated %P210 value is shown.
P210:percentage is 0.0000%	**P210 RNA was not detected or it is below the Limit of Detection** of the assay. Equivalent to %P210 = 0%.
Inconclusive - Retest Sample	**P210 RNA was detected but %P210 cannot be calculated.** Differences in P210 quantities within the duplicate are not acceptable. Retest the sample.
Invalid - Retest Sample	**ABL RNA was below the Cut-off (10,000 copies).** Retest the sample.

To complete the information for each sample analyzed, the results of single reactions (Tracks) for P210 and ABL targets are reported as follow.

Result of single Replicate	**Interpretation**
P210: RNA Detected, quantity equal to xxx copies/reaction	**P210 RNA was detected.** Calculated Quantity of mRNA of P210 is shown.
P210: RNA Not detected or below the LoD	**P210 RNA was not detected or it is below the Limit of Detection** of the assay.
ABL: RNA Detected, quantity equal to xxx copies/reaction	**ABL RNA was detected**. Calculated Quantity of mRNA of ABL is shown.
ABL: RNA Not detected or below the LoD	**ABL RNA was not detected or it is below the Limit of Detection** of the assay.

The following table that shows the different cases that might occur in an amplification session and the approach to generate the result messages.

Sample	P210 (copies/reaction)	ABL (copies/reaction)	Result of Sample run (%P210)	Interpretation
1st replicate	Quantity	Quantity ≥ 10,000	P210 percentage is x.xxxx%	**P210 RNA was detected.** Calculated %P210 value is shown.
2nd replicate	Quantity	Quantity ≥ 10,000		
1st replicate	Not Detected	Quantity ≥ 10,000	P210 RNA Not Detected or below the LoD	**P210 RNA was not detected or it is below the Limit of Detection** of the assay. Equivalent to %P210 = 0%
2nd replicate	Not Detected	Quantity ≥ 10,000		
1st replicate	Quantity < 10 copies	Quantity ≥ 10,000	P210 percentage is x.xxxx %	**P210 RNA was detected.** Calculated %P210 value is shown.
2nd replicate	Not Detected	Quantity ≥ 10,000		
1st replicate	Quantity > 10 copies	Quantity ≥ 10,000	Inconclusive-Retest Sample	**P210 RNA was detected but %P210 cannot be calculated.** Differences in P210 quantities within the duplicate are not acceptable. Retest the sample.
2nd replicate	Not Detected	Quantity ≥ 10,000		
1st replicate	Detected Or Not Detected	Quantity < 10,000	Invalid-Retest Sample	**ABL RNA was below the Cut-off (10,000 copies).** Retest the sample.
2nd replicate	Detected Or Not Detected	Quantity ≥ 10,000		
1st replicate	DETECTED Or NOT DETECTED	Quantity < 10,000	Invalid-Retest Sample	**ABL RNA was below the Cut-off (10,000 copies).** Retest the sample.
2nd replicate	DETECTED Or NOT DETECTED	Quantity < 10,000		

Note: if for a sample the result of the P210 amplification reaction is < 3 copies/reaction, the quantity will be reported to 3 copies/reaction.

Samples reported as "Invalid - Retest Sample" by the ELITe InGenius software are not suitable for result interpretation as the ABL mRNA was not detected efficiently. In this case, problems have occurred during the extraction phase (loss of RNA, presence of inhibitors or degradation of extracted RNA, see Troubleshooting) which may cause incorrect and false negative results. The sample is not suitable for calculation of %P210, the assay is invalid and must be repeated on extracted RNA first and, if a problem is confirmed, start from the extraction of a new sample.

Samples reported as "Inconclusive-Retest Sample" by the ELITe InGenius software are not suitable for result interpretation as the P210 mRNA was not detected efficiently. In this case, problems have occurred during the extraction phase (loss of RNA, presence of inhibitors or degradation of extracted RNA, see Troubleshooting) which may cause incorrect and false negative results. The sample is not suitable for calculation of %P210, the assay is inconclusive and must be repeated on extracted RNA first and, if a problem is confirmed, start from the extraction of a new sample.

Samples reported as "P210 RNA Not Detected or below LoD" are suitable for analysis but it was not possible to detect P210 RNA. In this case it cannot be excluded that the RNA is present at a concentration below the limit of detection of the assay (see "Performance characteristics").

Note: The results obtained with this assay must be interpreted taking into consideration all the clinical data and the other laboratory test outcomes concerning the patient.

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The Sample run results are stored in the database and, if valid, can be approved (Result Display) by personnel qualified as "Administrator" or "Analyst", following the GUI instruction. From the Result Display" window it is possible to print and save the Sample run results as "Sample Report" and "Track Report".

D. Samples result reporting

The sample results are stored in the database and can be viewed as "Sample Report" and "Track Report".

The "Sample Report" shows the details of a work session stored by selected sample (SID).

The "Track Report" shows the details of a work session by selected Track.

The "Sample Report" and "Track Report" can be printed and signed by authorized personnel.

PERFORMANCE CHARACTERISTICS

Limit of Detection

The P210 Limit of Detection of the assay with total RNA was verified using the reference calibrated material IVS10011 Clonal Control RNA (InVivoScribe, US), total RNA extracted from a human cell line positive for BCR-ABL P210 b3a2 diluted in total RNA from a human cell line negative for the translocation. The dilution 10^{-5} was tested in 20 replicates (300 ng of RNA / reaction), carrying out the reverse transcription and amplification reaction by ELITechGroup S.p.A. products in association with the ELITe InGenius system.

The final results are summed up in the following table.

Limit of Detection with total RNA samples and ELITe InGenius					
Sample	**Dilution**	**N**	**Positive**	**Negative**	**P210%**
P210 RNA	10^{-5}	20	20	0	0.0025%

All replicates resulted positive for P210, with a mean concentration of P210% equal to 0.0025%. The mean quantity of ABL recorded in the tests for the definition of the Limit of Detection was approximately 100,000 copies per reaction.

Linear measuring range

The P210 linear measuring range of this assay with total RNA was determined using the panel of reference calibrated material IVS10011 Clonal Control RNA (InVivoScribe, US). The panel consists of total RNA extracted from a human cell line positive for BCR-ABL P210 b3a2 diluted in total RNA from a human cell line negative for the translocation. The dilutions used ranged from pure P210 positive RNA (P210 RNA) to 10^{-5} (1 Log dilution steps). Each sample of the panel was tested in 4 replicates (300 ng of RNA / reaction), carrying out the reverse transcription and amplification reaction by ELITechGroup S.p.A. products in association with the ELITe InGenius system. The statistical analysis was performed by linear regression.

The analysis of the data obtained demonstrated that the assay has a linear response for the panel points from pure P210 positive RNA to 10^{-5} with a linear correlation coefficient greater than 0.99.

The upper limit of the linear measurement verified in this test is the pure P210 positive RNA, corresponding to a concentration of P210% equal to 100%.

The lower limit of the linear measurement verified in this test is the dilution of 10^{-5}, equal to the Limit of Detection and corresponding to a concentration of P210% equal to 0.0025%.

The final results are summed up in the following table.

Linear measuring range with total RNA samples and ELITe InGenius			
Sample	Mean P210 copies / reaction	Mean P210 Log copies /reaction	Std Dev
P210 RNA	474,505	5.676	0.02
$10^{-1.0}$ dilution	37,516	4.574	0.02
$10^{-2.0}$ dilution	3,545	3.549	0.02
$10^{-3.0}$ dilution	308	2.484	0.07
$10^{-4.0}$ dilution	36	1.553	0.06
$10^{-5.0}$ dilution	3	0.365	0.33

The mean quantity of ABL recorded in the tests was approximately 150,000 copies per reaction.

Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was tested analysing a panel of P210 positive clinical samples.

The diagnostic sensitivity was evaluated using 33 fresh specimens of peripheral blood collected in EDTA from leukemia patients tested positive for BCR-ABL translocation, variant P210 with a CE-IVD real time amplification product. Each sample was tested with **ELITe InGenius** in “Extract + PCR” mode.

The final results are summed up in the following table:

Samples	N	positive	negative	invalid
Peripheral blood samples positive for P210	33	32	1	0

In the test, 32 out of 33 samples were confirmed, one sample gave a discrepant negative result. In this test the diagnostic sensitivity of the assay was equal to 97%.

The mean quantity of ABL recorded in the tests was approximately 60,000 copies per reaction.

Diagnostic specificity: confirmation of negative samples

The diagnostic specificity of the assay, as confirmation of negative samples, was tested analysing a panel of P210 negative clinical samples.

The diagnostic specificity was evaluated using 41 fresh specimens of peripheral blood collected in EDTA from different subjects tested negative for BCR-ABL translocation, variant P210 with a CE-IVD real time amplification product. Each sample was tested with **ELITe InGenius** in “Extract + PCR” mode.

The final results are summed up in the following table:

Samples	N	positive	negative	invalid
Peripheral blood samples negative for P210	41	2	39	0

In the test, 39 out of 41 samples were confirmed, two samples gave a discrepant positive result. In this test the diagnostic specificity of the assay was equal to 95.1%.

The mean quantity of ABL recorded in the tests was approximately 50,000 copies per reaction.

N.B.: The complete data and results of the tests carried out to evaluate the performance characteristics of the product with matrices and instruments are recorded in the Product Technical File " BCR-ABL P210 ELITe MGB® Kit", FTP G07PLD210.

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ABI 7500 Fast Dx Real-Time PCR Instrument
ABI 7300 Real-Time System

SAMPLES AND CONTROLS

Samples

This product must be used with **RNA extracted** from the following clinical samples: lymphomonocyte and leukocyte suspensions from peripheral blood collected in EDTA or sodium citrate, bone marrow blood collected in EDTA or sodium citrate.

This product must be used adding up from 300 ng to 1.5 µg of **extracted RNA** to the reverse transcription and real time amplification reaction.

Suspensions of lymphomonocytes and leukocytes.

The suspensions of lymphomonocytes or leukocytes (e.g. buffy coat), used for RNA extraction, must be prepared from clinical samples of peripheral blood or bone marrow according to laboratory guidelines, resuspended in sterile physiological solution or sterile PBS and stored at +2 / 8 °C for a maximum of four hours.

The optimal quantity of lymphomonocytes or leukocytes from which to extract total RNA is approximately 10,000,000 cells.

Do not freeze suspensions of lymphomonocytes or leukocytes in order to avoid degradation of RNA.

The peripheral blood collected in EDTA or sodium citrate or bone marrow blood collected in EDTA or sodium citrate, used for lymphomonocytes or leukocytes preparation, must be collected according to laboratory guidelines, transported at +2 / 8 °C and stored at +2 / 8 °C for a maximum of four hours.

Do not freeze peripheral blood or bone marrow in order to prevent degradation of RNA.

Interfering substances

The extracted RNA must not contain heparin, haemoglobin, Ficoll®, ethanol or 2-propanol in order to prevent inhibition and frequent invalid results.

Quantities of RNA more than 1.5 µg per reaction could inhibit the reverse transcription reaction and the real time amplification.

Quantities of human genomic DNA higher than 100 ng per reaction in the RNA extracted from the sample could inhibit the reverse transcription reaction and the real time amplification.

There is no data available concerning inhibition caused by antibiotics, antiviral drugs, chemotherapeutic or immunosuppressant drugs.

Amplification controls

It is absolutely mandatory to validate each amplification session with a negative reaction control and a positive reaction control.

As a negative control (NC), use molecular biology grade water (not supplied with the product). This is to be added to the reaction in place of the RNA extracted from the sample.

As positive control (PC), the **«BCR ABL P210 ELITe Standard»** product is used.

Quality controls

It is recommended to validate the whole analysis procedure of each extraction, reverse transcription and amplification session by processing a negative sample and a positive sample that has previously been tested or reference calibrated material.

PROCEDURE

Setting of the real time amplification session
(To perform in the amplification / detection area)

Using **7300 Real-Time PCR System** or **7900 Real-Time PCR System** instrument.

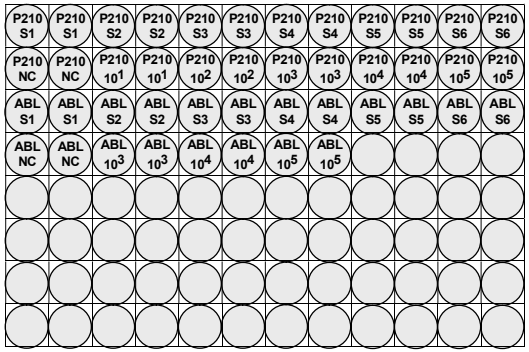
Before starting the session, referring to the instrument documentation, follow the instruction below:
- switch on the real time thermal cycler, switch on the control computer, launch the software and open an "absolute quantification" session,
- set (Detector Manager) the "detector" for the P210 probe with the “reporter” = "FAM" and the "quencher" = “none " (non fluorescent) " and name it "P210",
- set (Detector Manager) the "detector" for the ABL probe with the “reporter” = "FAM" and the "quencher" = “none” (non fluorescent) and name it "ABL",
- for each well in use in the microplate, set (Well Inspector) the "detector" (type of fluorescence to be measured), the "passive reference" = "ROX" (AP593 is used instead of ROX, normalisation of the measured fluorescence) and the type of reaction (sample, negative amplification control, positive amplification control or known quantity standard). Add this information to the **Work Sheet** enclosed at the end of this manual or print the microplate set up. The **Work Sheet** must be followed carefully during the transfer of the complete reaction mixture and samples into the wells.

Note: in order to determine the RNA titre in the starting sample, set up in duplicate the reactions with the **Q - PCR Standards** and the two complete reaction mixture to obtain the two standard curves, one for P210 (10^5, 10^4, 10^3, 10^2, 10^1 copies / reaction) and one for ABL (10^5, 10^4, 10^3 copies / reaction).

Note: To optimize the use of the product, the standard curve for P210 can be set up omitting the Q - PCR Standard level 10^1 copies / reaction and using the other 4 Q - PCR Standard levels (10^5, 10^4, 10^3, 10^2 copies / reaction) or using the Q - PCR Standard level 10^1 copies / reaction and omitting the Q - PCR Standard level 10^3 copies / reaction (10^5, 10^4, 10^2, 10^1 copies / reaction).

Note: calculate, for the target P210 and the control ABL, two wells for each sample to be analyzed (S), two wells for the negative control amplification (NC) and two wells for each Q - PCR Standard (5 or 4 points for P210 and 3 points for ABL).

Below is an example of how the analysis of 6 samples can be organized.



Key:

P210 S1 - **P210 S6**: P210 reactions with the test Samples,

P210 NC: P210 reaction with the Negative Control,

P210 10^1, **P210 10^2**, **P210 10^3**, **P210 10^4**, **P210 10^5**, P210 reactions with the 10^1, 10^2,10^3, 10^4 and 10^5 Q-PCR Standards.

ABL S1 - **ABL S6**: ABL reactions with the test Samples,

ABL NC: ABL reactions with the Negative Control,

ABL 10^3, **ABL 10^4**, **ABL 10^5**, ABL reactions with the 10^3, 10^4 and 10^5 Q-PCR Standards.

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Referring to the instrument documentation, set on the dedicated software (Instrument > Thermal Cycler Protocol > Thermal Profile) the parameters of the **thermal cycle**:

- add to the amplification stage a step (Add Step) for **extension at 72°C**,

Note: The fluorescence acquisition (Instrument > Thermal Cycler Protocol > Settings > Data Collection) must be set during the **hybridization at 56°C step**.

- modify timing as indicated in the table **"Thermal cycle"**,
- set the cycle number to **45**,
- set the reaction volume to **30 µL**.

Thermal cycle		
Phase	**Temperature**	**Time**
Reverse-transcription	50 °C	20 min.
Initial denaturation	94 °C	5 min.
Amplification and detection (45 cycles)	94 °C	10 sec.
	56 °C (fluorescence acquisition)	30 sec.
	72 °C	15 sec.

When **7500 Fast Dx Real-Time PCR Instrument** is used.

Before starting the session, referring to the instrument documentation, follow the instruction below:
- switch on the real time thermal cycler, switch on the control computer, launch the software, open an "absolute quantification" session and set "Run mode: Fast 7500",
- set (Detector Manager) the "detector" for the P210 probe with the “reporter” = "FAM" and the "quencher" = “none " (non fluorescent) " and name it "P210",
- set (Detector Manager) the "detector" for the ABL probe with the “reporter” = "FAM" and the "quencher" = “none” (non fluorescent) and name it "ABL",
- for each well in use in the microplate, set (Well Inspector) the "detector" (type of fluorescence that is to be measured), the "passive reference" = "CY5" (AP593 is used instead of CY5, normalisation of the measured fluorescence) and the type of reaction (sample, negative amplification control, positive amplification control or known quantity standard). Add this information to the **Work Sheet** enclosed at the end of this manual or print the microplate set up. The **Work Sheet** must be followed carefully during the transfer of the reaction mixture and samples into the wells.

Note: In order to determine the RNA titre in the starting sample, set up two series of reactions with the **Q - PCR standard** to obtain the two **standard curves**, one for P210 (10^5, 10^4, 10^3, 10^2, 10^1 copies / reaction) and one for ABL (10^5, 10^4, 10^3 copies / reaction).

Note: To optimize the use of the product, the standard curve for P210 can be set up omitting the Q - PCR Standard level 10^1 copies / reaction and using the other 4 Q - PCR Standard levels (10^5, 10^4, 10^3, 10^2 copies / reaction) or using the Q - PCR Standard level 10^1 copies / reaction and omitting the Q - PCR Standard level 10^3 copies / reaction (10^5, 10^4, 10^2, 10^1 copies / reaction)

Note: calculate, for the target P210 and the control ABL, two wells for each sample to be analyzed (S), two wells for the negative control amplification (NC) and two wells for each Q - PCR Standard (5 or 4 points for P210 and 3 points for ABL).

The setup of the quantitative analysis of 6 samples is shown, by way of example, in the previous paragraph describing the procedure for the **7300 Real Time PCR System** and **7900 Real Time PCR System** instruments.

Referring to the instrument documentation, set on the dedicated software (Instrument > Thermal Cycler Protocol > Thermal Profile) the parameters of the **thermal cycle**:

- add to the amplification stage a step (Add Step) for **extension step at 72 °C**,

Note: The fluorescence acquisition (Instrument > Thermal Cycler Protocol > Settings > Data Collection) must be set during the **hybridization step at 56 °C**.

- modify time as indicated in the table **"Thermal cycle"**,
- set the cycle number to **45**,
- set the reaction volume to **30 µL**.

Thermal cycle		
Phase	**Temperature**	**Time**
Reverse-transcription	50 °C	20 min.
Initial denaturation	94 °C	5 min.
Amplification and detection (45 cycles)	94 °C	10 sec.
	56 °C (fluorescence acquisition)	30 sec.
	72 °C	15 sec.

Amplification set-up
(To be performed in the extraction / preparation area)

Before starting the session, follow the instruction below:
- verify the availability of requested reagents for each sample to be analyzed (see table on page 10).
- remove and thaw at room temperature (+18 / 25 °C) the test tubes containing the RNA samples to be analysed. Vortex the tubes for 5 seconds, spin down the content for 5 seconds and keep them in a cold block,
- remove and thaw the **P210 PreMix** (WHITE cap) and **ABL PreMix** (NEUTRAL cap) test tubes needed for the session for 30 minutes at room temperature (+18 / 25 °C). Remember that the content of each test tube is enough for **50 reactions**. Vortex the tubes for 10 seconds three times, spin down the content for 5 seconds and keep them in a cold block,
- remove and thaw (+18 / 25 °C) the **PCR MasterMix** (NEUTRAL cap) tubes necessary for the session for 30 minutes at room temperature (+18 / 25 °C). Remember that the content of each tube is sufficient to set up **50** reactions. Vortex the tubes for 10 seconds three times, spin down the content for 5 seconds and keep them in a cold block,
- remove the **RT EnzymeMix** (BLACK cap) tubes necessary for the session remembering that the content of each tube is sufficient to set up **50 reactions**. Centrifuge for 5 seconds to bring the contents to the bottom and keep in a cold block,

Note: The **RT EnzymeMix** should not be exposed to temperatures above -20 °C for more than 10 minutes.

- remove and thaw the **P210-ABL Q-PCR Standard** tubes necessary for the session (**for both reactions P210 and ABL**) for 30 minutes at room temperature (+18 / 25 °C). Remember that the contents of each test tube are sufficient to set up **12 reactions**. Vortex the tubes for 10 seconds three times, spin down the content for 5 seconds and keep them in a cold block,
- take the **Amplification microplate** that will be used during the session, being careful to handle it with powderless gloves and not to damage the wells,
- take the **Amplification Sealing Sheet** that will be used during the session, being careful to handle it with powderless gloves and not to damage it,
- prepare two 1.5 mL sterile polypropylene tubes (not provided with this product): one for the complete reaction mixture of **P210** and the other for the complete reaction mixture **ABL** and mark them in a recognizable manner with a permanent marker,

- prepare two complete reaction mixtures, one for **P210** and the other for **ABL**, using the three components provided in the product, based on the number of samples to be analyzed, as described in the following table,

Note: For preparing one reverse transcription and real time amplification reaction 5 µL of PreMix, 15 µL of PCR MasterMix and 0,3 µL of RT EnzimeMix are needed. The volumes indicated in the table are sufficient for the setup of the reactions for reverse transcription and real time amplification required for the number of samples to be tested, negative control and Q-PCR Standard, in duplicate plus an adequate safety excess.

Number of samples	PreMix	PCR MasterMix	RT EnzymeMix
1	65 µL	195 µL	3.9 µL
2	75 µL	225 µL	4.5 µL
3	85 µL	255 µL	5.1 µL
4	95 µL	285 µL	5.7 µL
5	110 µL	330 µL	6.6 µL
6	120 µL	360 µL	7.2 µL
7	130 µL	390 µL	7.8 µL
8	140 µL	420 µL	8.4 µL
9	150 µL	450 µL	9.0 µL
10	160 µL	480 µL	9.6 µL
11	170 µL	510 µL	10.2 µL
12	180 µL	540 µL	10.8 µL
13	190 µL	570 µL	11.4 µL
14	205 µL	615 µL	12.3 µL
15	215 µL	645 µL	12.9 µL
16	225 µL	675 µL	13.5 µL
17	235 µL	705 µL	14.1 µL
18	245 µL	735 µL	14.7 µL
19	255 µL	765 µL	15.3 µL

Vortex the two complete reaction mixtures for 10 seconds three times, spin down the content for 5 seconds and keep them in a cold block.

Note: The complete reaction mixtures prepared should be used within 1 hour. The reaction mixtures prepared **cannot** be stored.

Set up **P210 and ABL reactions** as described below taking care to keep the **Amplification microplate** in a cold block (~ + 5 °C).

1. Accurately pipet **20 µL** of **"P210" complete reaction mixture** into the bottom of the **"P210" Amplification microplate** wells, as previously established in the **Work Sheet**. Avoid creating bubbles.
2. Accurately pipet **20 µL** of **"ABL" complete reaction mixture** into the bottom of the **"ABL" Amplification microplate** wells, as previously established in the **Work Sheet**. Avoid creating bubbles.
3. Accurately pipet **10 µL** of **RNA extract** into the complete reaction mixture from the first sample in the two corresponding wells of "**P210**" and in the two corresponding wells of "**ABL**" of the **Amplification microplate**, as previously established in the **Work Sheet**. Mix the sample well by pipetting the **extracted RNA** into the complete reaction mixture three times. Avoid creating bubbles both at the well bottom and in surface. Proceed in the same way with all the other samples of **extracted RNA**.
4. Accurately pipet **10 µL** of **Molecular biology grade water** (not provided with this product) into the complete reaction mixture in the two corresponding wells of "**P210**" and in the two corresponding wells of "**ABL**" of **Amplification microplate**, as previously established in the **Work Sheet**. Mix the negative control well by pipetting the **Molecular biology grade water** into the complete reaction mixture three times. Avoid creating bubbles both at the well bottom and in surface.

5. Accurately pipet **10 µL** of the first **P210-ABL Q-PCR Standard** into the complete reaction mixture in the two corresponding wells of "**P210**" of **Amplification microplate**, as previously established in the **Work Sheet**. Mix the standard well by pipetting the **P210-ABL Q-PCR Standard** into the complete reaction mixture three times. Avoid creating bubbles both at the well bottom and in surface. Proceed in the same way with the other **P210-ABL Q-PCR Standards**.
6. Accurately pipet, **10 µL** of the first **P210-ABL Q-PCR Standard** into the complete reaction mixture in the two corresponding wells of "**ABL**" of **Amplification microplate**, as previously established in the **Work Sheet**. Mix the standard well by pipetting the **P210-ABL Q-PCR Standard** into the complete reaction mixture three times. Avoid creating bubbles both at the well bottom and in surface. Proceed in the same way with the other **P210-ABL Q-PCR Standards**.
7. Accurately seal the **Amplification microplate** with the **Amplification Sealing Sheet**.
8. Transfer the **Amplification microplate** into the real time thermal cycler in the amplification / detection area and start the thermal cycle for the amplification saving the session setting with an univocal and recognizable file name (e.g. "year-month-day-BCR-ABL-P210-EGSpA").

Note: At the end of the thermal cycle the **Amplification microplate** containing the reaction products must be removed from the instrument and disposed of without producing environmental contamination. In order to avoid spilling the reaction products, the **Amplification Sealing Sheet must not be removed from the Amplification microplate**.

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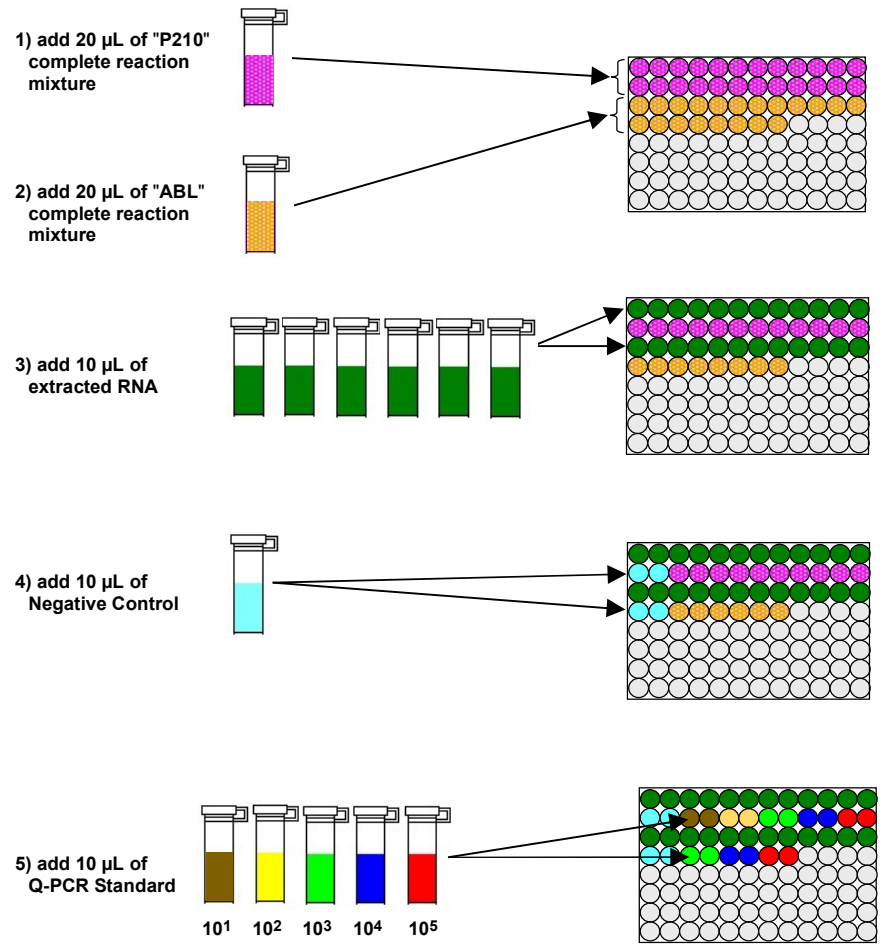
The following picture summarizes the setup of the reverse-transcription and real-time amplification reactions for P210 e ABL.



Analysis of the results

The values of fluorescence emitted by the specific probe for P210 (FAM detector "P210") in the P210 amplification reaction and by the specific probe for ABL (FAM detector "ABL") in the ABL amplification reaction must be analysed by the instrument software.

Before analysing, referring to the instrument documentation, it is necessary to:
- manually set (Results > Amplification plot > delta Rn vs Cycle) the calculation range for the **fluorescence background level (Baseline)** from cycle 6 to cycle 15,

Note: in the case of a positive sample with a high titre of P210 or ABL, the FAM fluorescence of the specific probe for P210 or ABL may begin to increase before the 15th cycle. In this case the calculation range for the "baseline" must be set for both detectors from cycle 6 to the cycle in which the FAM fluorescence starts to increase as detected from the instrument software (Results > Component).

- manually set the **Threshold** for the FAM detector "P210" to **0.1**,
- manually set the **Threshold** for the FAM detector "ABL" to **0.1**.

The values of fluorescence emitted by the specific probes in the amplification reactions and the **Threshold** value of fluorescence are used to determine the **Threshold Cycle (Ct)**, the cycle at which the fluorescence signal reaches the threshold value.

Standard Curve

In case of the P210 and ABL amplification reaction of the **Q - PCR Standards**, the **P210 and ABL Ct** values are used to calculate the two **Standard Curves** (Results > Standard Curve) of the amplification session and to validate the amplification and detection as shown in the following table:

P210 Reaction - Q - PCR Standard 10^5 detector FAM "P210"	Assay result	Amplification / Detection
Ct ≤ 25	POSITIVE	CORRECT

P210 Reaction - Standard Curve detector FAM "P210"	Acceptance range*	Amplification / Detection
Determination coefficient (R2)	0.970 ≤ R2 ≤ 1.000	CORRECT

ABL Reaction - PCR Standard 10^5 detector FAM "ABL"	Assay result	Amplification / Detection
Ct ≤ 25	POSITIVE	CORRECT

ABL Reaction - Standard Curve detector FAM "ABL"	Acceptance range	Amplification / Detection
Determination coefficient (R2)	0.990 ≤ R2 ≤ 1.000	CORRECT

***Note:** If the standard curve for P210 has been set up omitting Q - PCR Standard level 10^1 copies / reaction the acceptance range of the Determination Coefficient will be 0.990 ≤ R2 ≤ 1.000.

If the result of the **Q - PCR Standard 10^5** amplification reaction is **Ct > 25** or **Ct Undetermined** or if the **Determination coefficient** (**R2**) value does not fall within the limits, the target DNA has not been correctly detected. This means that problems occurred during the amplification or the detection step (incorrect preparation of the complete reaction mix, incorrect dispensing of the complete reaction mix or of the standards, degradation of the probe or of the standards, incorrect setting of the standard position, incorrect setting of the thermal cycle, see Troubleshooting), which may lead to incorrect results. The session is not valid and has to be repeated starting from the amplification step.

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Negative Control

In case of the P210 and ABL amplification reaction of the **Negative Control**, the **P210 and ABL Ct** values (Results > Report) are used to validate amplification and detection as shown in the following table:

P210 Reaction - Negative Control detector FAM "P210"	Assay result	Amplification / Detection
Ct Undetermined	NEGATIVE	CORRECT

ABL Reaction - Negative Control detector FAM "ABL"	Assay result	Amplification / Detection
Ct Undetermined	NEGATIVE	CORRECT

If the result of the **Negative control** amplification reaction is different than **Ct undetermined** for P210 and ABL, it means that target DNA has been detected in the amplification reaction. Problems have occurred during the amplification phase (contamination, incorrect preparation of the complete reaction mix, degradation of the probe, incorrect setting of the negative control position, incorrect setting of the thermal cycle, see Troubleshooting) which may cause incorrect results and false positives. The session is invalid and must be repeated from the amplification phase.

Samples

In case of the amplification reactions of each **sample**, **P210 Ct** values are used to detect and quantify the presence of target mRNA, while **ABL Ct** values are used to detect and quantify the presence of control mRNA (extraction validation and target normalization).

Note.: Verify by using the software tools of the instruments (Results > Amplification plot > delta Rn vs Cycle) that the **Ct** is determined by a rapid and regular increase of fluorescence values and not by isolated peaks or background signal increases.

The **P210 Ct** and **ABL Ct** values in the amplification reactions of each **sample** and the **Standard Curves** of the amplification session are used to calculate the **Quantity of mRNA** of P210 and ABL present in the amplification reactions of the samples.

Sample reactions		
Detector FAM	**mRNA**	**Quantity of mRNA obtained**
Ct determined	DETECTED	Quantity
Ct Undetermined	NOT DETECTED	0

The **Quantities** of the amplification reactions of **P210** and **ABL** for duplicates of each **sample** (Results > Report) are analysed as described in the following table that shows the different cases that might occur in an amplification session and the recommended approach to assess the data:

Sample	mRNA of P210	mRNA of ABL*	Calculated Quantity of mRNA of P210	Calculated Quantity of mRNA of ABL
1st replicate	DETECTED	Quantity ≥ 10,000	Sum Quantity	Sum Quantity
2nd replicate	DETECTED	Quantity ≥ 10,000		
1st replicate	NOT DETECTED	Quantity ≥ 10,000	0	Sum Quantity
2nd replicate	NOT DETECTED	Quantity ≥ 10,000		
1st replicate	Quantity < 10 copies	Quantity ≥ 10,000	Quantity	Sum Quantity
2nd replicate	NOT DETECTED	Quantity ≥ 10,000		
1st replicate	Quantity > 10 copies	Quantity ≥ 10,000	Retest the sample	
2nd replicate	NOT DETECTED	Quantity ≥ 10,000		
1st replicate	DETECTED or NOT DETECTED	Quantity < 10,000	Retest the sample	
2nd replicate	DETECTED or NOT DETECTED	Quantity ≥ 10,000		
1st replicate	DETECTED or NOT DETECTED	Quantity < 10,000	Retest the sample	
2nd replicate	DETECTED or NOT DETECTED	Quantity < 10,000		

*** Note:** If for a sample the result of the **ABL** amplification reactions is **ABL Quantity < 10,000** or **ABL NOT DETECTED** for at least one of the two replicates, this means that ABL mRNA was not detected efficiently. In this case, problems have occurred during the extraction phase (loss of RNA, presence of inhibitors or degradation of extracted RNA, see Troubleshooting) which may cause incorrect and false negative results.

Note: If for a sample the result of the amplification reactions is **P210 NOT DETECTED** and **ABL Quantity < 10,000** or **ABL NOT DETECTED** for at least one of two replicates, the result of assay is invalid and the sample is not suitable. The test must be repeated on extracted RNA first and, if the problem is confirmed, starting from the extraction of a new sample.

Note: If for a sample the result of the amplification reactions is **P210 DETECTED** and **ABL Quantity < 10,000** or **ABL NOT DETECTED** for at least one of two replicates, the result of assay is valid and the sample is positive for P210 mRNA. In this case, however, it is not possible to carry out the quantitative analysis. The test must be repeated on extracted RNA first and, if the problem is confirmed, starting from the extraction of a new sample.

Note: If the result of the amplification reaction of a sample is **P210 NOT DETECTED** and **ABL Quantity ≥ 10,000** for both replicates, the P210 mRNA has not been detected in the RNA obtained from the sample but it is not possible to exclude the presence of P210 mRNA at a lower titre than the detection limit of the product (see Performance Characteristics). In this case the result would be a false negative.

Note: If for a sample the result of the amplification reaction is **P210 Quantity > 10 copies** for one replicate and **P210 NOT DETECTED** for the other replicate and **ABL Quantity ≥ 10,000** for both replicates, the P210 mRNA has not been correctly detected in the RNA obtained from the sample. The result of assay is valid and the sample is positive for P210 mRNA. In this case, however, it is not possible to carry out the quantitative analysis. The test must be repeated on extracted RNA first and, if the problem is confirmed, starting from the extraction of a new sample.

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When the result of the amplification reactions of a sample is **P210 DETECTED** and **ABL Quantity ≥ 10,000**, the result of assay is valid, the sample is positive for P210 mRNA and it is possible to carry out the quantitative analysis.

The **calculated Quantities of P210 and ABL mRNA** of each sample are used to calculate the percentage of copies of P210 mRNA normalized to ABL mRNA copies (**P210 %**) in the starting sample according to this formula:

$$\text{P210 \%} = \frac{\text{Calculated Quantity of mRNA of P210}}{\text{Calculated Quantity of mRNA of ABL}} \times 100$$

The results obtained with this assay must be interpreted in consideration with all the clinical data and the other laboratory tests regarding the patient.

PERFORMANCE CHARACTERISTICS

Limit of Detection

The P210 Limit of Detection of the assay with total RNA was determined using a panel of dilutions prepared from reference calibrated material IVS10011 Clonal Control RNA (InVivoScribe, US). The panel consists of total RNA extracted from a human cell line positive for BCR-ABL P210 b3a2 diluted in total RNA from a human cell line negative for the translocation. The dilutions used ranged from $10^{-3.5}$ to 10^{-6} (0.5 Log dilution steps). Each sample of the panel was tested in 24 replicates (300 ng of RNA / reaction), carrying out the reverse transcription and amplification reaction by ELITechGroup S.p.A. products in association with the 7500 Fast Dx Real-Time PCR Instrument (Applied Biosystems). The statistical analysis was performed by Probit regression (SPSS 12.0.1). The limit of detection was defined as the dilution at which the probability of obtaining a positive result is equal to 95%.

The final results are summed up in the following table.

Detection limit with total RNA samples			
		Confidence interval of 95%	
		Lower limit	Upper limit
95% positivity	0.0016% P210% ($10^{-5.0}$ dilution)	($10^{-5.2}$ dilution)	($10^{-4.7}$ dilution)

The detection limit was defined at a dilution of $10^{-5.0}$ corresponding to a concentration of P210% equal to 0.0016%. The average amount of ABL recorded in the tests for the detection limit definition was approximately 200,000 copies per reaction.

Linear measuring range

The P210 linear measuring range of this assay with total RNA was determined using the panel of reference calibrated material IVS10011 Clonal Control RNA (InVivoScribe, US). The panel consists of total RNA extracted from a human cell line positive for BCR-ABL P210 b3a2 diluted in total RNA from a human cell line negative for the translocation. The dilutions used ranged from pure P210 positive RNA (P210 RNA) to 10^{-6} (1 Log dilution steps). Each sample of the panel was tested in 24 replicates (300 ng of RNA / reaction), carrying out the reverse transcription and amplification reaction by ELITechGroup S.p.A. products in association with the 7500 Fast Dx Real-Time PCR Instrument (Applied Biosystems). The statistical analysis was performed by linear regression (SigmaPlot 9.0).

The analysis of the data obtained demonstrated that the assay has a linear response for the panel points from pure P210 positive RNA to 10^{-5} with a linear correlation coefficient greater than 0.99.

The upper limit of the linear measurement verified in this test is the pure P210 positive RNA, corresponding to a concentration of P210% equal to 82.5%.

The lower limit of the linear measurement verified in this test is the dilution of 10^{-5}, equal to the Limit of Detection and corresponding to a concentration of P210% equal to 0.0016%.

The final results are summed up in the following table.

Linear measuring range with total RNA samples			
Sample	Mean P210 copies / reaction	Mean P210 Log copies /reaction	Std Dev
P210 RNA	358,276.92	5.55	0.04
$10^{-1.0}$ dilution	40,903.93	4.61	0.04
$10^{-2.0}$ dilution	4,150.86	3.62	0.04
$10^{-3.0}$ dilution	520.36	2.71	0.07
$10^{-4.0}$ dilution	59.02	1.76	0.11
$10^{-5.0}$ dilution	4.81	0.58	0.32

The mean quantity of ABL recorded in the tests for the definition of the linear measuring range was approximately 320,000 copies per reaction.

The measured quantity of P210 and ABL were verified using the European certified reference material ERM®-AD623 (IRMM, Belgium). The material consists of a dilution panel (1.0 Log dilution steps) of plasmid DNA containing P210 and ABL amplification products. The plasmid DNA concentration was calculated by digital PCR method. The dilutions used ranged from 10^6 copies / µL to 10^1 copies / µL. Each sample of the panel was tested in 9 replicates carrying out the amplification reaction by ELITechGroup S.p.A. products «BCR-ABL P210 ELITe MGB® Kit» and «BCR-ABL P210 ELITe Standard» in association with the 7500 Fast Dx Real-Time PCR Instrument (Applied Biosystems).

The data analysis, performed according to IRMM recommendations, showed that measurement of certified reference material obtained with ELITechGroup S.p.A. products are within the measurement uncertainty for quantities from 10^6 copies / µL to 10^1 copies / µL (equivalent to 10,000,000 copies per reaction and to 100 copies per reaction, by using 10 µL per reaction) for P210 target and from 10^6 copies / µL to 10^2 copies / µL (equivalent to 10,000,000 copies per reaction and to 1000 copies per reaction, by using 10 µL per reaction) for ABL target.

The final results are summed up in the following table.

Alignment of P210 measurement to the European reference material ERM®-AD623		
Certified Copies / µL	**Measured Copies / µL**	**Standard Deviation**
1,080,000	1,268,750	193,866
108,000	113,273	109,676
10,300	11,375	1,899
1,020	1,021	93
104	106	20
10.0	9.1	1.3

Alignment of ABL measurement to the European reference material ERM®-AD623		
Certified Copies / µL	**Measured Copies / µL**	**Standard Deviation**
1,080,000	1,355,000	197,990
108,000	129,250	12,781
10,300	13,427	1,843
1,020	1,150	140
104	116	17

Detection and quantification efficiency on possible polymorphisms

The analytical sensitivity of the assay, as the efficiency of detection and quantification with possible polymorphisms, was evaluated by comparison of sequences with nucleotide databases.

The verification of the hybridization regions of the primer oligonucleotides and of fluorescent probes (P210 and ABL) by alignment with the sequences of P210 and ABL human genes available in database showed their preservation and the absence of significant mutations.

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Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was tested analyzing a panel of P210 positive clinical samples.

The diagnostic sensitivity was evaluated using 49 archived RNA samples extracted from peripheral blood collected in EDTA or bone marrow blood samples obtained from leukemia patients tested positive for P210 with a CE-IVD real time amplification product. The samples were extracted with a method validated in the reference laboratory. The total extracted RNA (300 ng / reaction) reverse transcription and amplification reactions were carried out with ELITechGroup S.p.A. products on 7500 Fast Dx Real Time PCR Instrument.

The final results are summed up in the following table:

Samples	N.	positive	negative	
P210 Positive RNA from Whole Blood EDTA samples	49	49	0	

In this test the diagnostic sensitivity of the assay was equal to 100%.

The results on ABL Qty for peripheral blood or bone marrow blood samples were all above 40,000 copies/reaction.

Diagnostic specificity: confirmation of negative samples

The diagnostic specificity of the assay, as confirmation of negative samples, was tested analyzing a panel of P210 negative clinical samples.

The diagnostic specificity was evaluated using as 31 archived RNA samples extracted from peripheral blood collected in EDTA or bone marrow blood samples obtained from patients tested negative for P210 with a CE IVD amplification product. The samples were extracted with a method validated in the reference laboratory. The total extracted RNA (300 ng / reaction) reverse transcription and amplification reactions were carried out with ELITechGroup S.p.A. products on 7500 Fast Dx Real Time PCR Instrument.

The results are summed up in the following table:

Samples	N.	positive	negative	
P210 negative RNA from Whole Blood EDTA samples	31	0	31	

In this test the diagnostic specificity of the assay was equal to 100%.

The results on ABL Qty for peripheral blood or bone marrow blood samples were all above 20,000 copies/reaction.

N.B.: The complete data and results of the tests carried out to evaluate the performance characteristics of the product with matrices and instruments are recorded in the Product Technical File " BCR-ABL P210 ELITe MGB® Kit", FTP G07PLD210.

REFERENCES

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E. Beillard et al. (2003) *Leukemia* 17: 2474 - 2486
M. Baccarani et al. (2013) *Blood*: 122: 827 – 884
N. C. P. Cross et al. (2015) *Leukemia* 29: 999 - 1003
E. A. Lukhtanov et al. (2007) *Nucleic Acids Res.* 35: e30
F. Daraio et al. (2016) *Blood* 128: 5423

PROCEDURE LIMITATIONS

Only use RNA extracted with this product from the following clinical samples: suspensions of lymphomonocytes or leukocytes from peripheral blood collected in EDTA or citrate, bone marrow blood collected in EDTA or citrate.

Do not use RNA extracted from heparinized samples: heparin inhibits the reverse transcription and amplification reactions of nucleic acids and causes invalid results.

Do not use RNA contaminated by haemoglobin, dextran, Ficoll®, ethanol, or 2-propanol: these substances may inhibit the reverse transcription reaction and amplification reactions of the nucleic acids and cause invalid results.

Quantities of RNA more than 1.5 µg per reaction could inhibit the reverse transcription reaction and amplification reactions of the nucleic acids.

Do not use RNA with high quantities of human genomic DNA that can inhibit the reverse transcription and amplification reactions of nucleic acids and cause invalid results.

There is no data available concerning inhibition caused by antibiotics, antiviral drugs, chemotherapeutic drugs or immunosuppressant.

The results obtained with this product are subject to the correct identification, collection, transport, storage and preparation of samples. To avoid incorrect results, it is therefore necessary to take particular care during these phases and to carefully follow the instructions provided with the products for nucleic acid extraction.

Owing to its high analytical sensitivity, the real time amplification assay of nucleic acids used in this product is subject to contamination from clinical samples that are positive for P210, from positive controls and from the amplification reaction products themselves. Contamination leads to false positive results. The product has been designed in such a way as to reduce contamination; nevertheless, this phenomenon can only be prevented by following good laboratory practices and by complying scrupulously with the instructions provided in this manual.

This product must be handled by professional personnel qualified and trained in the processing of potentially infective biological samples and chemical preparations classified as dangerous to prevent accidents with potentially serious consequences for the user and other persons.

This product requires the use of work clothes and premises that are suitable for the processing of potentially infective biological samples and chemical preparations classified as dangerous to prevent accidents with potentially serious consequences for the user and other persons.

This product must be handled by professional personnel qualified and trained in molecular biology techniques, such as extraction, reverse transcription and amplification and detection of nucleic acids, to avoid incorrect results.

It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products to prevent false positive results.

This product requires the use of dedicated clothing and instruments for extraction/preparation of amplification reactions and for amplification/detection of amplification products to avoid false positive results.

Due to inherent differences between technologies, it is recommended that users perform method correlation studies to estimate technology differences prior to switching to a new technology.

A negative result obtained with this product means that the P210 mRNA is not detected in the reverse transcription reaction from the RNA extracted by the sample; but it cannot be excluded that the P210 mRNA has a lower titre than the product detection limit (see Performance Characteristics). In this case the result could be a false negative.

Results obtained with this product may sometimes be invalid due to inefficient detection of ABL mRNA and require retesting, starting from extraction, that can lead to a delay in obtaining final results.

Possible polymorphisms within the regions of the patient's genome covered by the product primers and probes may impair detection and quantification of P210 mRNA and ABL mRNA.

As with any diagnostic device, the results obtained with this product must be interpreted in consideration with of all the clinical data and other laboratory tests undertaken on the patient.

As with any diagnostic device, there is a residual risk of obtaining invalid results, false positives and false negatives with this product. This residual risk cannot be eliminated or reduced any further. In particular situations, this residual risk can contribute to incorrect decisions with potentially grave consequences for the patient.

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TROUBLESHOOTING

Target not detected in the Q - PCR Standard reactions or in the Positive control or invalid determination coefficient of the Standard curve	
Possible Causes	**Solutions**
Incorrect complete reaction mix preparation	Check the reagent volumes dispensed during complete reaction mix preparation
Incorrect dispensing into the microplate wells.	Take care when dispensing reagents into the microplate wells and comply with the work sheet. Check the volumes of complete reaction mix dispensed. Check the volumes of standard dispensed.
Incorrect session setup on ELITe InGenius	Check the position of reaction mixture, positive control or standards. Check the volumes of reaction mixture, positive control or standards.
Probe degradation.	Use a new aliquot of PreMix.
PCR MasterMix degradation.	Use a new aliquot of PCR MasterMix.
Positive control or Standard degradation.	Use a new aliquot of standard or positive control.
Instrument setting error.	Check the position settings for the standard reactions on the instrument. Check the thermal cycle settings on the instrument.
Instrument error.	Contact ELITechGroup Technical Service.

Target detected in the Negative control reaction	
Possible Causes	**Solutions**
Incorrect dispensing into the microplate wells.	Avoid spilling the contents of the sample test tube. Always change tips between one sample and another. Take care when dispensing samples, negative control and standards into the microplate wells and comply with the work sheet.
Incorrect session setup on ELITe InGenius	Check the position of reaction mixture or negative control. Check the volumes of reaction mixture or negative control.
Error while setting the instrument.	Check the position settings of the samples, negative control and standards on the instrument.
Microplate badly sealed.	Take care when sealing the microplate.
Contamination of the molecular biology grade water.	Use a new aliquot of water.
Contamination of the complete reaction mix.	Prepare a new aliquot of complete reaction mix.
Contamination of the extraction / preparation area for amplification reactions.	Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

Unexpected amplification profile of the target or target not detected in the sample reaction	
Possible Causes	**Solutions**
Incorrect complete reaction mix preparation.	Check the reagent volumes dispensed during complete reaction mix preparation; verify that RT EnzymeMix was added to complete reaction mix.
Incorrect dispensing into the microplate wells.	Avoid spilling the contents of the sample test tube. Always change tips between one sample and another. Take care when dispensing samples into the microplate wells and comply with the work sheet.
Incorrect session setup on ELITe InGenius	Check the position of reaction mixture or samples. Check the volumes of reaction mixture or samples.
Inhibition due to sample interfering substances.	Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a “PCR only” session. Repeat the extraction and amplification of sample performing a further washing step of the white cells pellet, to remove all red cells before lysis
RT EnzymeMix degradation.	Use a new aliquot of RT EnzymeMix.
Problems during reagent storage.	Verify that RT EnzymeMix was not exposed to temperatures higher than -20°C longer than 10 minutes. Verify that the complete complete reaction mix was not exposed to room temperature longer than 30 minutes.
Problems during extraction	Verify quality and concentration of extracted RNA.
Instrument error.	Contact ELITechGroup Technical Service.

Irregular or high background fluorescence in the reactions	
Possible causes	**Solutions**
Incorrect dispensing of sample.	Carefully mix by pipetting 3 times when adding samples, negative control and standards into the complete reaction mixture. Avoid creating bubbles both at the well bottom and in surface.
Baseline setting error.	Set the baseline calculation range the within cycles where the background fluorescence has already stabilized (check the "Results", "Component" data) and the signal fluorescence has not yet started to increase, e.g. from cycle 6 to cycle 15. Use the automatic baseline calculation by setting the "Auto Baseline" option.

Error 30103 on ELITe InGenius	
Possible causes	**Solutions**
Too high concentration of target in the sample.	If significant amplification is observed in PCR plot: - repeat the amplification with a 1:10 dilution in molecular biology grade water of eluted sample in a “PCR only” session or - repeat the extraction with a 1:10 dilution in molecular biology grade water of sample in an “Extract + PCR” session.

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REF **RTSG07PLD210**

SYMBOLS

REF Catalogue Number.

Upper limit of temperature.

LOT Batch code.

Use by (last day of month).

IVD *in vitro* diagnostic medical device.

Fulfilling the requirements of the European Directive 98\79\EC for *in vitro* diagnostic medical device.

Σ Contains sufficient for "N" tests.

Attention, consult instructions for use.

CONT Contents.

Keep away from sunlight.

Manufacturer.

NOTICE TO PURCHASER: LIMITED LICENSE

This product contains reagents manufactured by Life Technologies Corporation and are sold under licensing arrangements between ELITechGroup S.p.A. and its Affiliates and Life Technologies Corporation. The purchase price of this product includes limited, nontransferable rights to use only this amount of the product solely for activities of the purchaser which are directly related to human diagnostics. For information on purchasing a license to this product for purposes other than those stated above, contact Licensing Department, Life Technologies Corporation, 5781 Van Allen Way, Carlsbad, CA 92008. Phone: +1(760)603-7200. Fax: +1(760)602-6500. Email: outlicensing@thermofisher.com.

ELITe MGB® detection reagents are covered by one or more of U.S. Patents Nos 6,127,121, 6,485,906, 6,660,845, 6,699,975, 6,727,356, 6,790,945, 6,949,367, 6,972,328, 7,045,610, 7,319,022, 7,368,549, 7,381,818, 7,662,942, 7,671,218, 7,715,989, 7,723,038, 7,759,126, 7,767,834, 7,897,736, 8,008,522, 8,067,177, 8,969,003, RE 38,416 and EP patent numbers 0819133, 1068358, 1144429, 1232157, 1261616, 1430147, 1781675, 1789587, 1975256, 2714939 as well as applications that are currently pending.

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BCR-ABL P210 ELITe MGB® kit used with ELITe InGenius®

Code: RTSG07PLD210

This document is a simplified version of the official instruction for use. Please refer to the complete document before use: www.elitechgroup.com
This document is available only in English.

A. Intended use

The «BCR-ABL P210 ELITe MGB® Kit» product is a qualitative and quantitative, reverse transcription and amplification of nucleic acids assay for the detection of the mRNA of the BCR-ABL rearrangement, t(9;22) translocation, Philadelphia chromosome, variant P210 (P210) and for the quantification of the mRNA of P210 compared with the mRNA of the gene codifying the kinase protein Abelson (ABL). The assay is CE-IVD validated in combination with the instrument **ELITe InGenius®**.

B. Amplified sequence

	Gene	Fluorophore
Target	BCR-ABL (variant P210 b3a2 and P210 b2a2)	FAM
Control	ABL (exons a2a3)	FAM

C. Validated matrix

› **PBL isolated by buffycoat***

*Lympho-monocyte and/or leukocyte suspensions must be extracted from buffy coat from Peripheral Blood matrix

D. Kit content

P210 PreMix	ABL PreMix	PCR Master Mix	RT Enzyme Mix*
			
1 tube of 270 µL 36 reactions 6 freeze-thaw cycles **WHITE CAP**	1 tube of 270 µL 36 reactions 6 freeze-thaw cycles **NEUTRAL CAP**	2 tubes of 820 µL 36 reactions 6 freeze-thaw cycles per tube **NEUTRAL CAP**	2 tubes of 20 µL 36 reactions 6 freeze-thaw cycles per tube **BLACK CAP**

› Maximum shelf-life: 18 months

› 18 determinations in duplicate

› Storage Temperature: -20°C

** The RT EnzymeMix must not be exposed to temperatures higher than -20 °C for more than 10 minutes*

E. Material required not provided in the kit

› **ELITe InGenius instrument:** INT030
› **ELITe InGenius SP RNA:** INT034SPRNA
› **ELITe InGenius DNase I:** INT034DNASE
› **Dnase Tube Adapter Kit:** G6431-000
› **Cell Lysis Solution Promega*:** A7933
› **RNA Lysis Buffer Promega*:** Z3051
› **Thioglycerol Promega*:** A208B-C
** or equivalent*

› **ELITe InGenius PCR Cassette:** INT035PCR
› **ELITe InGenius SP200 Consumable Set:** INT032CS
› **BCR-ABL P210 - ELITe Positive Control:** CTRG07PLD210
› **BCR-ABL P210 ELITe Standard:** STDG07PLD210
› **PHILADELPHIA P210 RNA Reference:** SPG07PLD210
› **ELITe InGenius Waste Box:** F2102-000
› **300 µL Filter Tips Axygen:** TF-350-L-R-S
› **2 mL Sarstedt tube** :72.694.005

F. ELITe InGenius protocol

› Sample volume	200 µL
› Total eluate volume	100 µL
› PCR eluate input volume	10 µL for each PCR mix
› BCR-ABL Q-PCR Mix volume	20 µL for each PCR mix
› Report unitage	%P210
Frequency of controls	15 days
› Frequency of calibration	60 days

G. Sample pre-treatment

The sample need a blood pre-treatment to separate leukocyte by buffy- coat isolation, according to laboratory use or referring the indications shown in the “Samples and Controls” paragraph of the instruction for use.

H. Procedure

For the Calibration follow the table below:

Target	Number of Samples	PreMix	PCR MasterMix	RT EnzymeMix
P210	5	30 µL	90 µL	0.9 µL
ABL	3	20 µL	60 µL	0.6 µL

For Controls and samples follow the table below:

Number of Samples	P210 or ABL PreMix	PCR MasterMix	RT Enzyme Mix
1	15 µL	45 µL	0.5 µL
2	25 µL	75 µL	0.8 µL
3	40 µL	120 µL	1.2 µL

The complete reaction mixtures should be used within 5 hours when kept on board in the refrigerated block. This time allows to carry out 1 working session of 3.5 hours and to start a second working session. It's important to mix them between the runs. The complete reaction mixture **cannot be stored**.

I. Performance

Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
(PBL) Peripheral Blood Leukocyte	**0.0025%**	**97%** 32/33*	**95.1%** 39/41*

**confirmed samples/ tested samples*

J. Procedures

The user is guided step-by-step by the ELITe InGenius software to prepare the run. All the steps: extraction, reverse-transcription, amplification and result interpretation are automatically performed. Three operational modes are available: complete run, or extraction only, or PCR only.

Before analysis

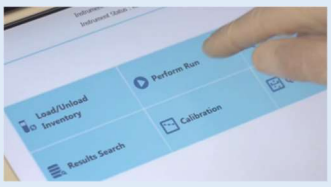
1. Switch on ELITe InGenius
Identification with username and password
Select the mode "Closed" or "Open"

Verify calibrators: BCR-ABL P210 Q-PCR Standard in the "Calibration menu". Verify controls: BCR-ABL P210 pos. and neg. controls in the "Control menu"
NB: Both have been run, approved and not expired

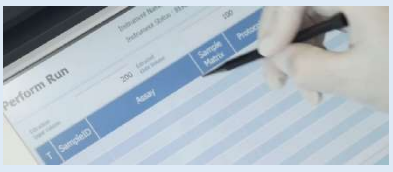
2. Thaw all the reagents and prepare 2 complete reaction mixture (P210 and ABL Mix) by adding into the dedicated 2 mL tube the calculated volumes of the three components for each Mix.
Mix by vortexing at low speed for 10 seconds three times, centrifuge the tube for 5 seconds
The complete reaction mixture should be used within **5** hours when kept on board in the refrigerated block

Procedure 1 - Complete run: Extraction + PCR

1. Select "Perform Run" on the touch screen



2. Verify the extraction volumes.
Input: "200 µL", eluate: "100 µL"



3. Scan the sample barcodes with hand-held barcode reader or type the sample ID

4. Select the “Assay protocol BCR-ABL P210 ELITe_PBL_200_100”

5. Select the sample position: sonication tube

6. Load the complete reaction mixture on the “Inventory Block”

7. Load: PCR cassette, the ELITe InGenius SP RNA extraction cartridges, the ELITe InGenius DNase I and all the required consumables

8. Close the door
Start the run

9. View, approve and store the results

Procedure 2 - PCR only

1 to 4: Follow the Complete Run procedure described above	**5.** Select the protocol “PCR only” and set the sample position “Elution tube”	**6.** Load the extracted nucleic acid tubes in the rack n°4
7. Load the PCR cassette rack Load the complete reaction mixture in the inventory block	**8.** Close the door Start the run	**9.** View, approve and store the results

Procedure 3 - Extraction only

1 to 4: Follow the Complete Run procedure described above	**5.** Select the protocol “Extraction Only” and set the sample position: sonication tube	**6.** Load: the ELITe InGenius SP RNA extraction cartridges, the ELITe InGenius DNase I and all the required consumables
7. Close the door Start the run	**8.** Archive the eluate sample	

BCR-ABL P210 ELITe MGB® Kit used with ABI PCR instrument

Code: RTSG07PLD210

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EMPOWERING IVD

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A. Intended use

The BCR-ABL P210 ELITe MGB Kit is a qualitative and quantitative, reverse transcription and amplification of nucleic acids assay for the detection of the mRNA of the BCR-ABL rearrangement, t(9;22) translocation, *Philadelphia* chromosome, variant P210 (P210) and for the quantification of the mRNA of P210 compared with the mRNA of the gene codifying the kinase protein Abelson (ABL). The assay is CE-IVD validated in combination with ABI PCR thermal cyclers (Thermo-Fisher) and and laboratory validated extraction system such as the «Maxwell® CSC» (Promega) automatic extraction system or other equivalent products.

Amplified sequence

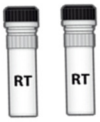
	Gene	Fluorophore
Target	**BCR-ABL rearrangement (variant P210 b3a2 and variant P210 b2a2)**	FAM
Internal Control	**ABL (exons a2a3)**	FAM

B. Validated matrix

› **Peripheral blood collected in EDTA or sodium citrate or bone marrow***

*Lympho-monocyte and/or leukocyte suspensions must be extracted from matrices mentioned above

C. Kit content

P210 PreMix	ABL PreMix	PCR Master Mix	RT Enzyme Mix*
			
1 tube of 270 µL 50 reactions 6 freeze-thaw cycles **WHITE CAP**	1 tube of 270 µL 50 reactions 6 freeze-thaw cycles **NEUTRAL CAP**	2 tubes of 820 µL 50 reactions 6 freeze-thaw cycles per tube **NEUTRAL CAP**	2 tubes of 20 µL 50 reactions 6 freeze-thaw cycles per tube **CAP with BLACK INSERT**

› Maximum shelf-life: 18 months 25 reactions in duplicate › Storage Temperature: -20°C

** The RT Enzyme Mix must not be exposed to temperatures higher than -20 °C for more than 10 minutes*

D. Material required not provided in the kit

› **Maxwell® CSC:** AS6000
› **7500 Fast Dx, 7300 and 7900 PCR Instrument**
› **BCR-ABL P210 ELITe Standard:** STDG07PLD210
› **BCR-ABL P210 - ELITe Positive Control:** CTRG07PLD210
› **PHILADELPHIA P210 RNA Reference:** SPG07PLD210
› **Molecular biology grade water**

E. Performance

System	Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
Maxwell - ABI	Peripheral blood or bone marrow	**0,0016% P210%** $10^{-5.0}$ Dilution	**100%** (49/49)	**100%** (31/31)

F. Procedure

The procedure below summarized the main steps of the sample analysis with conventional PCR workflow: validated extraction systems, PCR instrument settings, PCR set-up and result interpretation.

Complete reaction mixtures reconstitution

1. Thaw P210 PreMix and ABL PreMIx, PCR MasterMix, vortex 10 sec 3 times, spin down 5 sec
2. RT Enzyme Mix should not be exposed to T° > -20°C more than 10min. Gently shake, spin down 5 sec
3. Prepare two 1.5 ml tube, one for the complete reaction mixture of P210 and the other for complete reaction mixture ABL
4. Calculate the required volume of the 3 components for each complete reaction mixture

Note: The volumes indicated in the table are sufficient for the setup of the reactions for reverse transcription and real time amplification required for the number of samples to be tested, negative control and Q-PCR Standard, in duplicate plus an adequate safety excess.

Samples	PreMix	PCR MasterMix	RT Enzyme Mix
1	65 µL	195 µL	3,9 µL
2	75 µL	225 µL	4,5 µL
3	85 µL	255 µL	5,1 µL
4	95 µL	285 µL	5,7 µL
5	110 µL	330 µL	6,6 µL
6	120 µL	360 µL	7,2 µL
7	130 µL	390 µL	7,8 µL
8	140 µL	420 µL	8,4 µL
9	150 µL	450 µL	9,0 µL
10	160 µL	480 µL	9,6 µL
11	170 µL	510 µL	10,2 µL
12	180 µL	540 µL	10,8 µL
13	190 µL	570 µL	11,4 µL
14	205 µL	615 µL	12,3 µL
15	215 µL	645 µL	12,9 µL
16	225 µL	675 µL	13,5 µL
17	235 µL	705 µL	14,1 µL
18	245 µL	735 µL	14,7 µL
19	255 µL	765 µL	15,3 µL

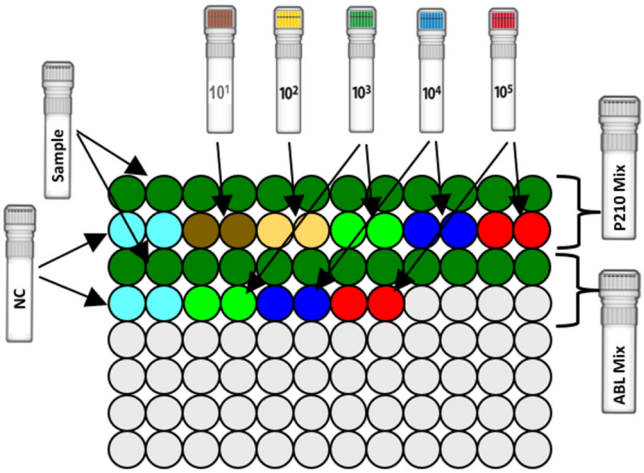
Amplification - Settings of 7500 Fast Dx and 7300, 7900 PCR instruments

1. Switch on the thermal-cycler
2. Set “P210” detector with “FAM” and quencher “none”
3. Set “ABL” detector with “FAM” and quencher “none”
4. Set passive reference as “Cy5” with 7500 Fast Dx and as “ROX” with 7300, 7900 instruments
5. Set up the thermal profile as indicated. Fluorescence acquisition must be set during hybridization step at 56°C

Stage	Temperature	Timing
Reverse Transcription	50°C	20 min
Initial Denaturation	94°C	5 min
Amplification and detection *45 cycles*	94°C 56°C 72°C	10 sec 30 sec 15 sec

Amplification - PCR Set-up

1. Thaw BCR-ABL P210 Q-PCR standard tubes
2. Mix gently and spin-down
3. Prepare the “P210 PCR MIx” and “ABL PCR MIx” by adding the required volume of three components as reported in table above. The complete reaction mixture should be used within 30 min and cannot be stored
4. Pipet 20 µL of “P210 PCR-Mix after reconstitution in all microplate wells in use
5. Pipet 20 µL of “ABL PCR Mix” after reconstitution in all microplate wells in use
6. Add, 10 µL of extracted RNA in sample wells, 10 µL of molecular grade water in Negative Control well, and 10 µL of the 5 Q-PCR Standards in standard curve wells
7. Extracted RNA samples, Q-PCR Standards and Negative Control must be pipet in duplicate
8. Seal the microplate with the amplification sealing sheet
9. Transfer the microplate in the thermocycler and start



Amplification - Threshold for quantitative analysis

Instrument	P210 FAM	ABL FAM
7500 Fast Dx Real Time PCR	0.1	0.1
7300 and 7900 Real Time PCR	0.1	0.1

Interpretation - quantitative results

Detector FAM	mRNA	Quantity of mRNA
Ct determinated	Detected	Quantity
Ct Undetermined	**Not detected**	**0**

P210 Ct value Neg-Control	Results	Amplification/Detection
Ct Undetermined	Negative	Correct
ABL Ct value Neg-Control	**Results**	**Amplification/Detection**
Ct Undetermined	Negative	Correct

Sample	mRNA of P210	mRNA of ABL	Calculated Quantity of mRNA of P210	Calculated Quantity of mRNA of ABL
1st replicate	**DETECTED**	**Quantity ≥ 10,000**	**Sum Quantity**	**Sum Quantity**
2nd replicate	**DETECTED**	**Quantity ≥ 10,000**		
1st replicate	**NOT DETECTED**	**Quantity ≥ 10,000**	**0**	**Sum Quantity**
2nd replicate	**NOT DETECTED**	**Quantity ≥ 10,000**		
1st replicate	**Quantity < 10 copies**	**Quantity ≥ 10,000**	**Quantity**	**Sum Quantity**
2nd replicate	**NOT DETECTED**	**Quantity ≥ 10,000**		
1st replicate	**Quantity > 10 copies**	**Quantity ≥ 10,000**	**Retest the sample**	
2nd replicate	**NOT DETECTED**	**Quantity ≥ 10,000**		
1st replicate	**DETECTED or NOT DETECTED**	**Quantity < 10,000**	**Retest the sample**	
2nd replicate	**DETECTED or NOT DETECTED**	**Quantity ≥ 10,000**		
1st replicate	**DETECTED or NOT DETECTED**	**Quantity < 10,000**	**Retest the sample**	
2nd replicate	**DETECTED or NOT DETECTED**	**Quantity < 10,000**		

Percentage of copies of P210 mRNA normalized to ABL mRNA copies (P210 %)

Detector FAM	mRNA	P210 %
P210 Ct determinated	Detected	**Calculated Quantity of mRNA of P210** ——————————————————— **x 100** **Calculated Quantity of mRNA of ABL**
ABL Ct determinated	Detected (**Quantity ≥ 10,000**)	